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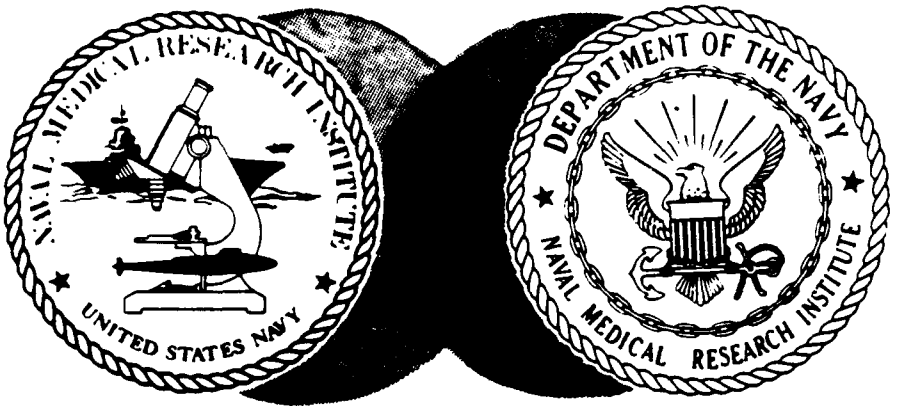

80-91

EFFECT OF ENDOTOXIN ON OXYGEN CONSUMPTION BY A FLOW-CONTROLLED CANINE HIND-LIMB PREPARATION

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The effect of endotoxin upon oxygen consumption in a peripheral vascular bed was assessed in a canine hind-limb preparation. The left iliac vessels in 12 adult male mongrel dogs were isolated, the artery cannulated proximally and distally such that arterial inflow could be routed through a roller pump, and monocclusive sampling catheter placed in the vein. Oxygen consumption was then calculated as the product of flow rate and arteriovenous oxygen content difference at a series of flow rates before and after the systemic administration of endotoxin (3 mg/kg) or saline solution. (cont'd)

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Item 20 continued:

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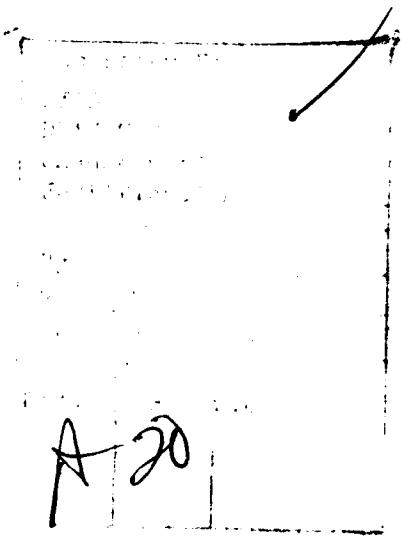

A-20

EFFECT OF ENDOTOXIN ON OXYGEN CONSUMPTION BY A FLOW-CONTROLLED CANINE HIND-LIMB PREPARATION

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Effect of endotoxin on oxygen consumption by a flow-controlled canine hind-limb preparation

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The effect of endotoxin upon oxygen consumption in a peripheral vascular bed was assessed in a canine hind-limb preparation. The left iliac vessels in 12 adult male mongrel dogs were isolated, the artery cannulated proximally and distally such that arterial inflow could be routed through a roller pump, and a nonocclusive sampling catheter placed in the vein. Oxygen consumption was then calculated as the product of flow rate and arteriovenous oxygen content difference at a series of flow rates before and after the systemic administration of endotoxin (3 mg/kg) or saline solution. At each flow rate, oxygen consumption by the extremities of endotoxic animals was significantly greater ($P < 0.01$) than that measured in control animals. This finding mitigates the hypothesis that endotoxin has a direct effect on cells reducing tissue oxygen consumption.

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SEPTIC SHOCK in man is characterized by depression of the peripheral vascular resistance, narrowing of the central arteriovenous oxygen difference, and in some instances, elevation of the cardiac output.[2, 12, 17] Conversely, cardiogenic and hypovolemic shock are characterized by a reduction in the cardiac output and a widening of the central arteriovenous oxygen difference.[11] This has caused MacLean[10] to formulate a unified definition of shock as "inadequate blood flow to vital organs or the inability of the body cell mass to metabolize nutrients normally." Four explanations for the narrowed arterial-mixed venous oxygen difference encountered in clinical septic shock might be proposed: (1) arterial oxygen saturation is insufficient to deliver oxygen to the tissues, (2) the blood flow somehow bypasses the metabolic units of the periphery, (3) the red blood cell or microvascular channels are altered such that oxygen is not released to the tissues, or (4) the cells of the peripheral tissues are altered such that they are unable to utilize the oxygen available to them.

The first statement, that arterial oxygen content is insufficient to supply peripheral needs, is belied by the usual finding of a normal or elevated arterial Po_2 in the early phases of septic shock. Indeed, the use of the hyperbaric chamber to increase arterial Po_2 to abnormally high levels has been shown to have no effect on the narrowed arteriovenous difference.[12]

Proponents of the second statement postulate the opening of precapillary arteriovenous shunts as explanation of both the failure of peripheral oxygen utilization and the reduction in peripheral resistance. Cronenwett and Lindenauer[3] have recently claimed to demonstrate the presence of such shunts in a septic canine hind limb. According to the technique of Hedenreck and Thal,[7] fecal-soaked strips of umbilical tape were implanted in the calf, thigh, and paw of the hind limb of each of six dogs. Seventy-two hours later, blood flow, arteriovenous

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oxygen difference, and arteriovenous shunting were measured and compared with similar measurements in a control group of animals. With this technique, Cronenwett and Lindenauer[9] demonstrated a 5-fold increase in arteriovenous shunting in the septic limb which they felt correlated significantly with the decreased arteriovenous oxygen difference across the extremity. It must be recognized, however, that these data were obtained across a focus of acute inflammation and are therefore not precisely interpretable as a basis for the systemic changes in septic shock. Other investigators, using preparations challenged by a systemic septic insult, have been unable to demonstrate such shunts. Wright et al.[18] measured cardiac output, hind-limb muscle blood flow, and arteriovenous oxygen difference in a canine peritonitis model devised by Clowes et al.[4] With the demonstration of a strong correlation between the increase in cardiac output and a commensurate increase in muscle capillary blood flow, Wright et al.[18] concluded that arteriovenous shunting was not present and could not, therefore, provide an explanation for the narrowed arteriovenous oxygen difference. Utilizing a radioactive microsphere technique, Archie[1] was similarly unable to demonstrate anatomic arteriovenous shunting in either endotoxic or septic canine shock preparations.

In the third statement a defect in oxygen transport or diffusion is proposed. Archie,[1] in explaining his negative results, suggested that impaired oxygen diffusion or "physiologic shunting" might be the cause of the reduced arteriovenous oxygen difference in septic shock. The regulation of hemoglobin affinity for oxygen provides one such avenue for impairment. It is well known that the intraerythrocytic concentration of 2,3-diphosphoglycerate (2,3-DPG) can alter hemoglobin oxygen affinity and the position of the oxyhemoglobin dissociation curve.[13] Depression in 2,3-DPG causes an increase in the oxygen affinity of hemoglobin, or a leftward shift of the oxyhemoglobin dissociation curve, thereby decreasing peripheral oxygen extraction and narrowing the central arteriovenous oxygen difference. A leftward shift is favored by a low pH, and acidosis is a hallmark of both clinical and experimental septic and endotoxic shock. Miller et al.,[15] in 1970, was able to measure depressed 2,3-DPG levels in patients with septic shock and found that these values correlated with the position of the oxyhemoglobin dissociation curves. The authors also indicated that the depressed 2,3-DPG levels correlated with the narrowing of the arteriovenous oxygen difference. More recent experimental studies have not confirmed a consistent relationship among endotoxin, 2,3-DPG, and oxygen extraction. Naylor et al.[16] measured p50, 2,3-DPG, and oxygen consumption in Rhesus monkeys exposed to endotoxin. They were unable to detect systematic changes in any one of the three measurements from control levels and concluded that it was unlikely that 2,3-DPG–related changes in p50 play a major role in augmenting oxygen delivery in experimental shock. In a somewhat different study, Garg et al.[6] examined splanchnic blood flow and oxygen consumption from 2,3-DPG–rich fresh blood vs. 2,3-DPG–poor 21-day-old blood in dogs subjected to a 2 mg/kg dose of Difco endotoxin. Although the two perfusates differed significantly in 2,3-DPG content, oxygen consumption from the two products did not. These authors concluded that 2,3-DPG depletion did not impair oxygen extraction by tissues exposed to endotoxin. Broadie and Herman,[3] in a canine hind-limb preparation, were similarly unable to show improved oxygen consumption from 2,3-DPG–rich fresh vs. 2,3-DPG–depleted 21-day-old blood. The bulk of evidence now suggests that 2,3-DPG levels and hemoglobin affinity for oxygen are insufficient to explain the defects in oxygen extraction purported to be present in clinical sepsis.

The fourth proposal states that the tissues themselves are altered such that they are no longer able to consume oxygen. This concept is enunciated as a corollary to MacLean's definition[10] of shock in which septic shock is described as "a primary cellular defect characterized by low oxygen utilization." The finding by Wright et al. of depressed muscle oxygen consumption in the absence of arteriovenous shunting in a septic canine preparation was interpreted as evidence for a cellular utilization defect. Mela et al.[14] examined rat liver mitochondria harvested at graded time intervals after the induction of endotoxin shock. They were able to demonstrate impaired respiratory activity and ascribed this to progressive failure of membrane structure. Harken, Lillo, and Hufnagel[8] more recently have studied the oxygen consumption of rabbit liver slices and cell-free rabbit liver homogenates exposed to *Escherichia coli* endotoxin. They found that the oxygen consumption of each preparation was depressed on exposure to endotoxin and inferred that endotoxin has the capacity to depress, directly, cellular respiration. On the basis of data derived from the cell-free homogenates, they concluded that the effect was mediated on subcellular systems and that the intact cell membrane conferred

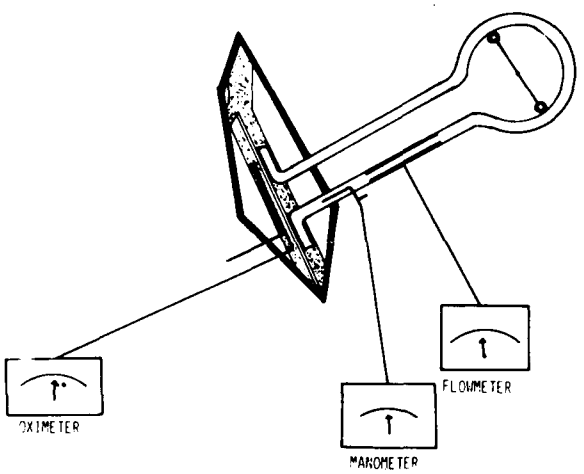

Fig. 1. Diagram of the surgical preparation showing placement of arterial catheters and arterial and venous monitoring devices.

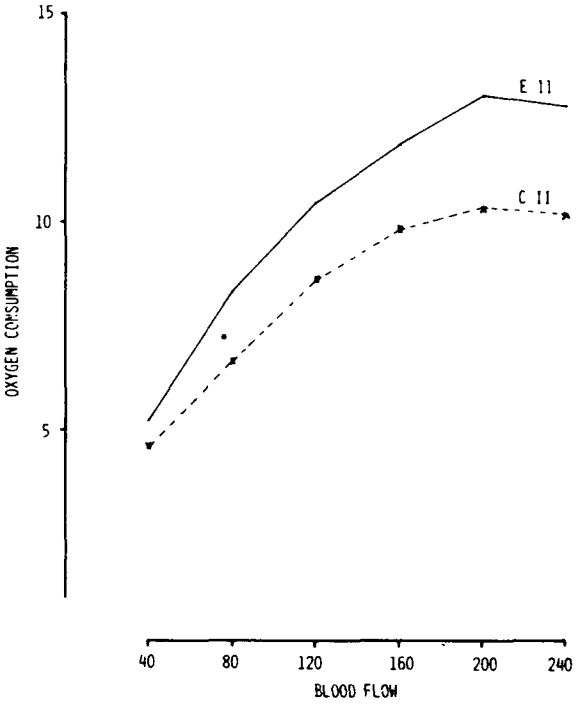

Fig. 2. Mean oxygen consumption (ml O_2/min) of the left hind limb plotted against left femoral arterial blood flow (ml/min) for the second cycles of the endotoxin group (*E II*, n = 6) and the control group (*C II*, n = 6). The separation between the two curves is significant at the $P < 0.01$ level of confidence.

no protection to these structures. These data suggest that endotoxin does have the capacity to affect directly the respiratory mechanisms of individual cells over and above any effects related to altered flow and reduction in substrate availability. Some caution in the interpretation of Harken's work is warranted by considerations of the equivalency of the dose employed in these in vitro studies to the dose employed in whole-animal preparations.

The data supporting each explanation for the concept of septic shock as a state of inadequate oxygen uptake are then not entirely consistent. As suggested by Gump,[7] the concept of a narrowed arteriovenous oxygen difference and reduced oxygen consumption in sepsis may even be artifactual. He correctly notes the large variance in many studies and suggests that some of the findings may simply be based upon anemia. From the relationship,

$$\Delta AV_{O_2} = 0.0134\ H\ (Sa_{O_2} - Sv_{O_2}) + 0.0031\ (Pa_{O_2} - Pv_{O_2})$$

where

ΔAV_{O_2} = Arteriovenous oxygen difference
H = Hemoglobin concentration
Sa_{O_2}, Sv_{O_2} = Arterial and venous oxygen saturations
Pa_{O_2}, Pv_{O_2} = Arterial and venous oxygen tensions

it is apparent that a reduction in hemoglobin will reduce the ΔAV_{O_2}. Gump[7] presents a series of patients in whom an elevated cardiac output and *apparent* reduced arteriovenous oxygen difference were maintained, but in whom the mixed venous oxygen saturation remained unchanged; the difference was explained readily by the reduction in hemoglobin concentration. Given this confusion, the present study utilizing a canine hind-limb preparation was undertaken to determine whether, in fact, endotoxin could cause a diminution in oxygen consumption in a peripheral vascular bed in which rate of inflow would not be a limiting factor.

MATERIAL AND METHODS

Twelve conditioned, adult male mongrel dogs weighing between 20 and 25 kg were anesthetized with intravenous pentobarbital (3 mg/kg), intubated, and placed in a supine position on a small-animal operating table. Through a small cervical incision, the right carotid artery was cannulated, and by means of a strain-gauge manometer and recorder, systemic arterial blood pressure was continuously measured.

A sigmoid incision was then made in the left groin and the inguinal ligament divided to expose the iliac vessels in the retroperitoneal space. The iliac and common femoral arteries were dissected free for their entire length from the aortic bifurcation to the takeoff of the profunda femoris artery; the internal

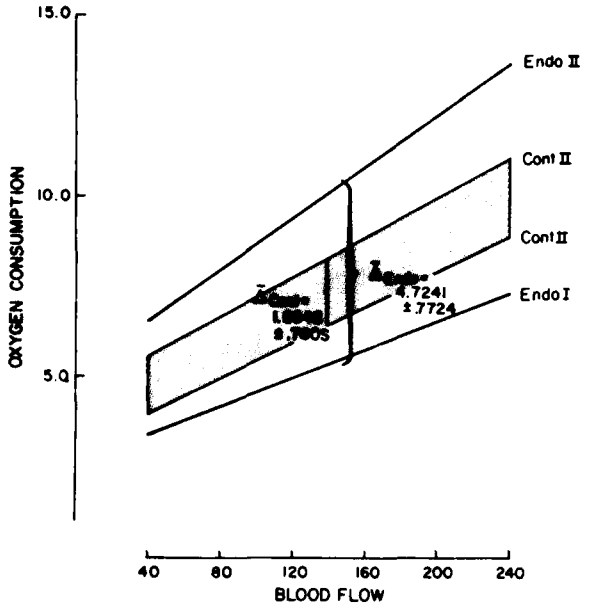

Fig. 3. Regression of oxygen consumption on flow for the control group cycles I and II (*cont I* and *cont II*) and the endotoxin group cycles I and II (*endo I* and *endo II*), with graphical representation of Δ_{endo} and Δ_{cont}.

iliac artery was ligated. The external iliac vein was similarly isolated, and the internal iliac vein doubly ligated and divided. A nonoccluding sampling catheter was directed into the femoral vein such that its tip lay at the level of the inguinal ligament. An Edwards Laboratories Oximeter Catheter (Model 01-020) was then passed via the saphenous vein into the femoral vein such that its tip lay 1 to 2 cm cephalad to the tip of the sampling catheter (Fig. 1).

After completion of the surgical preparation, the animal was heparinized (heparin, 2 mg/kg), and two No. 16 to No. 18 French cannulas were placed in the external iliac artery. The first was directed proximally so as to lie within the abdominal aorta. The second was placed such that its tip lay immediately proximal to the division of the common femoral artery into the deep and superficial femoral arteries. The two cannulas were then connected by a saline-filled length of ¼ inch Tygon tubing containing the inline transducer of an electromagnetic flow probe (Fig. 1).

The tubing was directed through the pump head of a roller pump proximal to the flow transducer (Fig. 1). The preparation thus afforded a means of controlling the rate of arterial inflow and sampling access to the venous effluent.

The animal was allowed to breathe spontaneously

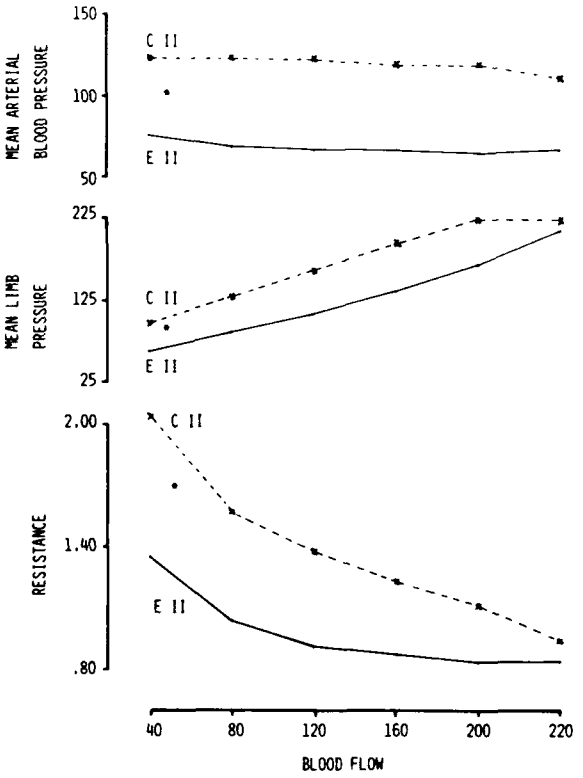

Fig. 4. Mean systemic arterial blood pressure (mm Hg), mean limb arterial blood pressure (mm Hg), and limb vascular resistance (mm Hg · min · ml^{-1}) plotted against left femoral arterial blood flow (ml/min) for the second cycles of the endotoxin group and the control group.

*Differences between groups significant at the $P < 0.005$ level of confidence.

from a gas-mixing apparatus, a mixture of N_2 and sufficient O_2 to maintain arterial oxygen saturation at or near 100% throughout the course of the experiment as assured by intermittent sampling of arterial oxygen content, blood gases, and hemoglobin. An adequate level of anesthesia was maintained by periodic supplemental doses of pentobarbital. Heparin (1 mg/kg) was given at hourly intervals to maintain anticoagulation. Body temperature was kept near 38° C by means of a heating pad and heat lamp. Hydration was maintained throughout the lengthy course of the experiment by the continuous intravenous infusion of sterile normal saline solution.

In each experiment, oxygen consumption by the extremity was measured at several rates of flow, varying in 40 ml/min increments from 40 to 240 ml/min. A 5-minute equilibration period was allowed at a given rate of flow before data were

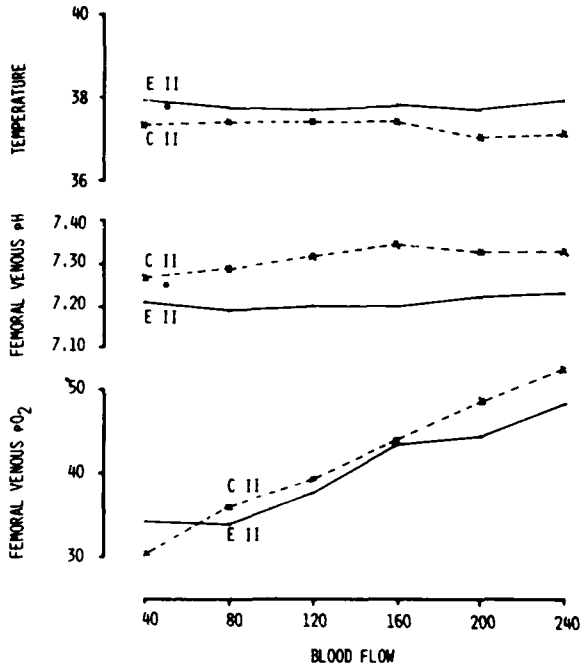

Fig. 5. Mean limb temperature (°C), left femoral venous pH, and left femoral venous Po_2 (mm Hg) plotted against left femoral arterial blood flow for the second cycles of the endotoxin group *(E II)* and the control *(C II)*.

*Differences between groups significant at the $P < 0.005$ level of confidence.

collected and recorded. Each experiment consisted of two such cycles separated by a period of 30 minutes. At the completion of the first cycle, each animal was randomly assigned to one of two groups, such that at the end of the project six animals had been assigned each to the control group and the experimental group. Immediately at the conclusion of the first cycle, each animal in the experimental group was given Difco endotoxin in a dose of 3 mg/kg (diluted in saline solution to 10 mg/ml) intravenously; this has been determined to be a LD_{50} dose in our laboratory. Those randomized to the control group received a volume of diluent similarly calculated on a weight basis.

At each data collection point, blood was drawn from the femoral vein for determination of oxygen content, plt, Po_2, CO_2, and hemoglobin concentration; and limb temperature, venous oxygen saturation, arterial blood pressure, and arterial blood flow were recorded. By manipulation of the oxygen content, Po_2, and hemoglobin concentration with the following relationship, a calculated value for venous or arterial oxygen saturation was determined:

$$S_{O_2} = \left[\frac{Cx_{O_2} - 0.0031Po_2}{1.34\ H}\right](100)$$

where

S_{O_2} = % Oxygen saturation
Cx_{O_2} = Oxygen content (mlO_2/100 ml blood)
H = Hemoglobin concentration (gm/100 ml blood)
0.0031 = Conversion factor in units (ml O_2/100 ml blood · mm Hg)

The three arterial saturations in each cycle were pooled and a mean determined. A linear regression of venous oxygen saturations was then drawn against the simultaneously recorded oximeter readings of venous oxygen saturation, and from the regression equation, a corrected value for venous oxygen saturation for each flow rate was calculated. Using this corrected value for venous oxygen saturation and the mean arterial oxygen saturation in each cycle, oxygen consumption at each flow rate was then calculated from the following equation:

$$\dot{V}_{O_2} = F\left[0.0134\ (\bar{S}a_{O_2} - Sv_{O_2}) + 0.0031\ (\bar{P}a_{O_2} - Pv_{O_2})\right]$$

Data were collated by group, cycle, and flow rate and compared; the significance of differences between groups in each cycle was then ascertained by two-way analysis of variance. An alternative mode of analysis was applied to the oxygen consumption data. In each experiment a linear regression of oxygen consumption vs. flow was drawn for each cycle, and the average distance, Δ, between the two lines within the limits of 40 and 240 ml/min were determined. Values for Δ were then pooled within the control and experimental groups and a mean, $\bar{\Delta}$, and standard error of the mean for each group were determined. The means were then compared and the validity of the comparison was ascertained by Student's t test.

RESULTS

The oxygen consumption data are listed in Table I. The mean consumption rates for each flow in the second cycles of the endotoxin (E II) and control groups (C II) are displayed graphically in Fig. 2. At each flow rate, mean oxygen consumption by the hind limb is seen to be greater in the group exposed to endotoxin than in the comparable control group. This increase is significant at the $P < 0.01$ level of confidence. The increase in oxygen consumption with flow rate in each group is also significant at the $P < 0.01$ level of confidence.

Table I. Oxygen consumption in the experimental periods (E II and C II) and baseline periods (E I and C I) of preparations given endotoxin (E) and saline (S)

Period	*Ex. No.*	*40*	*80*	*120*	*160*	*200*	*240*
	1	5.7	8.3	11.5	9.1	11.0	10.6
	2	7.0	11.7	11.6	13.5	13.7	15.4
E II	3	4.3	6.8	8.6	10.7	4.5	8.3
	9	5.6	7.5	8.5	11.3	11.6	10.2
	11	4.3	7.7	10.4	12.6	16.0	15.7
	13	4.9	8.3	12.2	14.0	15.8	11.2
$\bar{X}_E$ II		5.3	8.4	10.5	11.9	12.1	11.9
	5	7.1	8.7	9.5	10.0	10.0	11.3
	6	4.1	6.2	8.1	9.0	8.5	7.3
C II	7	4.1	5.2	7.0	7.4	8.3	9.6
	8	3.2	6.0	8.5	9.8	9.7	8.6
	11	4.8	6.6	8.4	10.2	11.0	10.1
	14	5.1	7.5	10.4	13.1	15.0	14.0
$\bar{X}_C$ II		4.7	6.7	8.7	9.9	10.4	10.2
	1	4.0	7.1	5.2	6.0	7.1	10.2
	2	4.7	5.7	7.2	13.0	7.7	13.0
E I	3	3.0	3.8	3.4	5.6	4.5	8.3
	9	4.3	5.0	4.7	4.6	4.4	4.9
	11	2.1	4.4	5.1	3.0	3.0	4.9
	13	1.9	3.3	4.2	5.2	4.1	4.5
$\bar{X}_E$ I		3.3	4.9	5.0	6.2	5.1	7.6
	5	5.6	6.4	8.4	9.4	12.2	13.6
	6	3.0	3.7	3.8	4.8	4.3	5.5
C I	7	4.7	7.1	7.9	7.5	9.9	10.2
	8	2.1	4.1	5.0	6.0	7.3	7.6
	11	3.1	3.6	4.6	4.7	4.6	5.8
	14	3.8	6.5	8.0	9.4	11.2	9.2
$\bar{X}_C$ I		3.7	5.2	6.3	7.0	8.3	8.7

NOTE: The increase in mean oxygen consumption at each flow rate in E II relative to C II is significant at the $P < 0.01$ level of confidence. The increase in oxygen consumption with flow rate in each group in both periods is also significant at the $P < 0.01$ level of confidence.

Calculation of the average separation, Δ, between the regressions of oxygen consumption on flow for cycles I and II of each experiment provides an alternate mode of analysis. This approach allows the use of each animal as its own baseline, and on this basis tends to minimize individual variation when values are pooled within the two groups. This information is summarized graphically in Fig. 3. It is apparent that the average separation in the endotoxin group, $\bar{\Delta}_{endo} = 4.7241 \pm 0.7724$, is approximately 2.5 times the separation in the control group, $\bar{\Delta}_{cont} = 1.8645 \pm 0.7605$. This difference is significant at the $P < 0.05$ level of confidence.

The separation between the curves representing the first and second cycles in the control group indicates some adaptation to the experimental situation which affords or obligates an increase in oxygen consumption even in the control situation. The finding that the magnitude of this separation is significantly greater in the group exposed to endotoxin supports the conclusion that there is present a significant increase in oxygen consumption by the tissues of the hind limb of the animals exposed to endotoxin as compared to those not so exposed.

Table II presents mean systemic arterial limb pressure, mean arterial blood pressure, limb resistance, temperature, femoral venous pH, and femoral venous Po_2 data collated by group and flow rate. Mean systemic arterial blood pressure (MABP) fell, venous pH decreased, and temperature rose in those animals exposed to endotoxin (Figs. 4 and 5). The arterial pressure in the perfused extremity is also depressed in the endotoxin group at each flow rate relative to the comparable control value, as is vascular resistance. These changes (E II vs. C II) are significant at the $P < 0.005$ level of confidence and document the degree of the endotoxic challenge.

Femoral venous Po_2 in the endotoxin group was

Table II. Mean systemic blood pressure, limb blood pressure, limb vascular resistance, limb temperature, and femoral venous pH and Po_2 tabulated by flow rate and group

Flow (ml/mm)	*Period*	*40*	*80*	*120*	*160*	*200*	*240*	*E II vs C II*	*Flow*
Mean systemic arterial	E II	75	69	66	66	64	66	$P < 0.005$	NS
Blood pressure (mm Hg)	C II	123	123	122	119	118	111		
Mean arterial pressure	E II	63	85	108	140	169	208	$P < 0.005$	$P < 0.005$
in limb (mm Hg)	C II	98	129	165	195	223	224		
Resistajce in liib	E II	1.35	1.03	0.91	0.87	0.84	0.84	$P < 0.005$	$P < 0.005$
(mm Hg. min.ml $^{-1}$)	C II	2.02	1.57	1.38	1.22	1.11	0.94		
Temperature of	E II	37.9	37.7	37.7	37.8	37.7	37.9	$P < 0$*005	NS
limb (°C)	C II	33.3	37.2	37.4	37.4	37.0	37.1		
Femoral venous	E II	34.2	34.0	37.8	43.3	44.5	48.5	NS	$P < 0.005$
Po_2 (mm Hg)	C II	30.3	36.0	39.2	43.8	48.7	52.5		
Femoral venous	E II	7.21	7.19	7.20	7.20	7.22	7.23	$P < 0.005$	NS
pH	C II	7.27	7.29	7.32	7.35	7.33	7.33		

not significantly less than that in the control group, but venous Po_2 in both groups increased significantly with increasing rate of arterial inflow, implying both increased extraction at lower flow rates in both groups and continued variable extraction rates in the presence of endotoxemia.

DISCUSSION

The results of this experiment indicate that oxygen consumption by an intact canine hind limb preparation is increased upon challenge of the organism with endotoxin in a LD_{50} dose. This information runs counter to the prevailing prejudice that septic shock is a state in which there is present a defect in peripheral oxygen utilization. As indicated, however, this latter concept is predicated largely upon central arteriovenous oxygen differences determined in septic patients and upon in vitro analyses of the function of subcellular organelles; both approaches are subject to interpretive license. An increase in oxygen extraction in the face of increased energy demands by the peripheral tissues is certainly more appropriate to homeostasis. Such a response might be postulated as compensation for the reduction in oxygen availability occasioned by the reduction in flow.

The other data collected in the experiment fail to provide insight into the mechanism(s) by which the increase in oxygen consumption might occur. The temperature and pH changes are too small to cause an alteration in hemoglobin oxygen affinity sufficient to produce a change of the magnitude seen, although the changes seen are in the appropriate direction. Although 2,3-DPG was not measured, the duration of the experiment in this case seems too short to implicate changes in 2,3-DPG concentration. The drop in resistance might be construed as evidence for precapillary shunts, but this would not explain increased consumption. Overall, the explanation does not seem to lie in mechanisms involved in the transport of oxygen to the extremity. It is emphasized that in this preparation, the hind limb was isolated only in that the rate of arterial inflow could be controlled and the venous effluent sampled; the extremity was otherwise intact and subject to systemic neurologic and humoral control mechanisms. Any of the humoral mediators of cellular metabolism mobilized by an endotoxin challenge might be responsible for the increase in oxygen consumption. Alternatively, a more direct effect of endotoxin upon the cells themselves might be responsible. While we would favor the former suggestion, the design of the experiment does not allow resolution of such speculation.

CONCLUSIONS

Oxygen consumption by a flow-controlled canine hind-limb preparation is increased significantly upon challenge of the organisms by endotoxin. The explanation for this effect does not appear to lie among the determinants in oxygen delivery.

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DISCUSSION

Dr. Clowes (Boston, Mass.). I think it is very nice to see a study of one part of the problem of septic shock, but it does bring up a number of really difficult things to consider.

First, what is septic shock? I think we have to keep separate in our minds what sepsis does. The typical septic patient who is doing well has a high cardiac output and a low peripheral resistance. On the other hand, when the cardiovascular system is incapable of satisfying this big circulatory demand, septic shock is present.

There are three major causes for this circulatory insufficiency. One is the loss of fluid from the circulation, or hypovolemia. The second is the increase of pulmonary vascular resistance that may lead to right heart failure. The third is myocardial depression.

We have had the opportunity to do a number of studies comparing high-output and low-output sepsis. We found that in the septic high-output patient, oxygen consumption in the periphery is up and glucose consumption is up. Lactate production is huge. In the low-output state in which vasoconstriction takes place as the circulation fails, we see reduced peripheral consumption, the lactate production is further increased as glucose uptake goes further up. The breakdown of muscle protein proceeds at an even greater pace.

I want to ask a question: Is endotoxin really a good model for studying septic shock as it occurs clinically? Two things must be brought out. The first is that when endotoxin is given expermentally there is an initial phase of hypotension. After 1, 2, or 3 hours normotension returns, and 24 hours later there is a gradual progression of falling blood pressure. The secondary hypotention is accompanied by a marked reduction in blood glucose. In other words, the endotoxin has seriously damaged the liver, and glucogenesis in this organ fails. Dr. Tom O'Donnell, working with us, studied this pehnomenon and came to the conclusion that endotoxin shock is a poor model for studying human sepsis.

On the matter of what endotoxin does in relation to blood, We find that Lena Mela's work and ours show that endotoxin by itself affects tissues very differently from when it is exposed with the presence of blood. This is an old observation.

In any case, we are not surprised to see that the oxygen consumption rises in these particular studies simply because the circulation was artifically maintained at a high rate, in contrast to what happens clinically.

I think these are important observations on the effects of endotoxin on muscle tissue in an intact organism, even though they are difficult to interpret.

Dr. Charles Wright (Saskatoon, Saskatchewan, Canada). My comments echo those of Dr. Clowes in many ways. This is an elegant study and it addresses itself to an interesting question, but I am not certain this study has answered the question.

The authors addressed the question of septic shock but the model deals with endotoxin shock, and there are some very major differences between these two. There are major differences in the effects of endotoxin in different species; there are major differences in the effects of sepsis as opposed to that of endotoxin in the same species; gram-positive organisms contain no endotoxin whatever but are responsible for an identical clinical syndrome in approximately 50% of cases nowadays. The key issue of course is that it is completely impossible in a model using endotox-

emia to duplicate the hyperdynamic phase which is seen in clinical septic shock. This model cannot duplicate the septic shock model by simply giving endotoxin and then producing an increased circulation to the local part of the limb.

I have two brief questions: First, was the flow pulsatile? This might be another issue in this argument. Second, why did the authors not incorporate one of the several septic models which are available into their model, together with the flow studies, rather than using that of endotoxemia, which is of very doubtful relevance?

Dr. John R. Border (Buffalo, N.Y.). I think we must stop speaking of septic shock. There is no septic shock. There is a shock associated with sepsis which has multiple mechanisms that change with time. There are grave doubts as to how much endotoxin has to do with the septic physiologic metabolic changes in man. There have been millions of dollars spent on endotoxin in animals. It kills dogs very nicely, and that says absolutely nothing about sepsis in man.

We recently published a report on a series of patients showing different organisms—gram positive, gram negative, *Bacteroides,* fungi—and as far as we could tell all had the same circulatory changes and the same metabolic changes, independent of whether or not the organism contained endotoxin.

I have grave doubts about endotoxin and its involvement in the septic changes that occur in man. I think this study helps me to have more of those doubts. Clearly the things that have been said in the past about its reducing oxygen consumption, per se, are not seen in this study. I suspect that is true in most of the things we see in clinical sepsis.

Dr. Thomas A. Broadie (closing). I would like to thank Dr. Clowes, Dr. Wright, and Dr. Border for their comments. I am sure I will be unable to answer their basic problem, which is whether or not endotoxin is a fitting measure analogue to clinical septic shock. This question probably is unresolvable at the present time.

In response to Dr. Wright's specific question as to whether flow was pulsatile . . . it was not. We did use a roller pump. We used this model because this was an acute experiment. Most of the other models are really unsuited to the way we had designed this experiment.

Our approach was to measure a specific event which has been recorded in clinical sepsis, that is, the supposed reduction in oxygen consumption by cells and the reasons for this. We had anticipated that there would be a reduction and were surprised at our findings.

We have done other experiments of a not dissimilar nature in a totally isolated hind-limb preparation which were reported in *Surgical Forum* last year; in that situation there was no change in oxygen consumption. This is an area that does deserve some further look.